

A BACTERIOLOGICAL STUDY OF
DECOMPOSING CRABS AND
CRAB MEAT

A Dissertation

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS
UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

1930

BY

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BALTIMORE

1932



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(Received for publication June 8, 1931.)

Introduction.

Although crab meat is a highly perishable article of food, practically no literature bearing on a bacteriological study of its decomposition has been found. The lack of such investigations in this country at least, is undoubtedly due to the fact that crabs and crab meat have not been implicated in the spread of such diseases as typhoid fever. In China, however, crabs have been definitely proven to be a vehicle for the spread of cholera since in that country they not only live and breed in polluted waters, but are often eaten in the raw state.

In Maryland, and it is undoubtedly true throughout this country where crabs can be obtained, the whole living crab (usually soft crab) when bought by the housewife is boiled for fifteen minutes or half an hour before it is in any way used as food, while crab meat is obtained from hard crabs that have been steamed in one way or another for half an hour, more or less. This boiling of the soft crab and steaming of the hard crab probably kills most, if not all, pathogenic bacteria that may be present.

Crab cakes, however, have frequently been involved by the laity as the cause of intestinal disturbances, sometimes of a grave nature, and since little has been published on the bacterial content of crab meat which is used to make up these "cakes" a bacteriological study of crab decomposition may be of some value. The chief object in pursuing this investigation has been to determine the manner in which crabs and crab meat decompose; and to arrive at some conclusions as to the role of bacteria in bringing about such changes.

Review of the literature.

Buttenberg (10) states that crabs in the shell decompose by bacterial action; no attempts were made however to identify and

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determine the organisms responsible for the spoilage. He noted that crabs decomposing in the shell have an ammoniacal "fishy" odor and as spoilage progresses further a "putrid" odor (Fäulniss).

An analysis of the gases produced in crabs decomposing in the shell showed the following:

Oxygen	0- 2.8 per cent
Hydrogen	0- 1.8 " "
Nitrogen	27.0-58.9 " "
Carbon dioxide	33.1-67.2 " "
Sulphur trioxide	0-13.0 " "

He also states that decomposition can be brought about by non-gas producing bacteria and that as soon as bacteria in crab meat multiply the ammonia content rises rapidly, even though the crab meat may look fresh. Buttenberg's studies appear to be the only ones in which bacteriological examinations of crabs are reported.

Experimental.

Samples. About eighty pounds of crab meat, equivalent to the meat obtained from approximately two hundred crabs, were examined during the course of this study. This included both Maryland and Virginia crab meat. Maryland crab meat was not more than twenty-four hours old when purchased while the Virginia meat was probably not more than forty-eight hours old. Whole live crabs when they could be obtained in season were also studied. The live crabs were purchased on the markets, brought to the laboratory, and were killed either by placing them in a glass jar containing ether or chloroform, or by bleeding them, inserting a sterile pointed probe into the heart.

Aerobic technique. The sample of crab meat when brought to the laboratory was immediately transferred from the carton in which it was sold on the market to a chemically clean, sterile Mason pint jar with a clamp top. It was found that these jars equipped with rubber between the lid and jar proper when sterilized in the autoclave for 15 minutes at 15 pounds pressure at 120° C. could not be opened easily due to the fact that the heat made the top stick to the rubber. Replacing the rubber with gauze eliminated this difficulty. Before sterilization chemically clean glass beads were placed in the jars. Ten grams of the sample to be studied were placed in a sterile jar, 90 cc. of sterile, neutral distilled water added and then the whole shaken on a mechanical shaker for ten minutes. One cc. samples of this suspension, considered as a 1-10 dilution, were used in making further dilutions. Throughout the entire study plates were always made in triplicate.

The agar plates were incubated at 20° C. and 37° C., while gelatin plates were incubated at room temperature, 18–20° C. Counts were made after 48 hours incubation. During the course of this investigation it was found that by far the greatest number of bacteria grew best at 20° C.

On several samples total counts were made by the dilution method as well as by plating. This method may be briefly described as follows: All dilutions were made and planted in nutrient broth. Four samples of each dilution were used and incubated at 20° C. or 37° C. for 48 hours. A sufficient number of dilutions were made so that all of the tubes containing the highest dilution were sterile. Results were recorded by placing in a series the number of positive results secured in each set of dilutions. The *significant number* was then determined, i.e., the figures representing the highest dilution in which all tubes were positive, and the next two. The most *probable number* of bacteria is then found with reference to the particular *significant number* obtained. The most probable number is the number of bacteria present in the dilution which corresponds to the first figure of the significant number. Multiplying the most probable number by this dilution gives the number of bacteria per cubic centimeter or gram. (For a more detailed discussion of obtaining total counts by this method consult Buchanan and Fulmer, 1928, vol. I.)

When the whole crab was to be studied it was killed as described, scrubbed with a stiff brush under running tap water, flamed several times with alcohol, and the dorsal shell, carapace, carefully cut away with a sterile pair of scissors so as not to injure the underlying organs and muscle meat. The stomach and intestines were removed and triturated in a sterile mortar with 18 cc. of 2 per cent saline and then shaken on a mechanical shaker for ten minutes. This was considered a 1–10 dilution. The other internal organs including the liver, ovaries, heart, and muscle meat were triturated in a sterile mortar with 90 cc. of sterile 2 per cent saline and then shaken for ten minutes on the mechanical shaker. From this dilution of the two separate portions further dilutions were made. Plates in triplicate were incubated both at 37° C. and at room temperature.

The usual differential media were used for the identification of individual organisms. Plain beef extract 2 per cent agar and 18 per cent gelatin were used for plates and tubes. Endo's litmus lactose, eosin-methylene-blue agar and Eijkman's dextrose broth were used for the isolation of *Bacillus coli*. The fluorescent organisms were cultivated on crab infusion agar and the streptococci on both blood

and crab infusion agar. Dextrose litmus agar slants and plates were used for certain dextrose fermenting forms. Growth and liquefaction were observed on Loeffler's coagulated blood serum slants and in gelatin while chromogenesis was observed on potato. Litmus milk, plain beef extract broth and infusion broth were also used. One per cent carbohydrate media with Andrade as the indicator were used for testing acid and gas production. The carbohydrates regularly employed were: dextrose, levulose, galactose, arabinose, xylose, inosite, sucrose, lactose, maltose, raffinose, starch, dextrin, glycogen, inulin, glycerol, dulcitol, and mannitol. Other special media and tests included Koser's citrate and uric acid media, reduction of nitrates, indol production, hydrogen sulphide production, methyl red reaction, Voges-Proskauer reaction, gas formula, and ammonia production as tested with Nessler's reagent. All tests were performed where practicable according to the "Standard Methods" of the Society of American Bacteriologists.

Anaerobic technique. For the isolation of anaerobes two portions of undiluted material of about one to two grams each were heated at 56° C. for twenty minutes for one portion and at 80° C. for ten minutes for the other and inoculated into cooked meat tubes and sealed with vaseline. All anaerobic media were boiled for fifteen minutes to expel the dissolved oxygen and then cooled quickly by placing in ice water before inoculation. Duplicates were also made with unheated material. High milk tubes were inoculated for the attempted detection of *Clostridium welchii*. After two to five days incubation material was withdrawn from the meat tubes and shaken in sterile 2 per cent saline with sand for ten minutes. Shake tubes were then made of this material and isolated colonies fished to freshly boiled meat tubes.

Crab spoilage. Most of the crabs kept at ice box temperature showed no perceptible signs of spoilage up to the fifth or ninth day. The carapace was sound and was only removed with some difficulty. The internal organs and muscle meat appeared fresh and solid and had a pleasant "fishy" odor. Different samples varied in the time at which definite signs of spoilage were evident. The perceptible signs of spoilage in order of their appearance seemed to be as follows: (1), a strong "fishy" odor, (2), carapace easily removed, (3), a still stronger disagreeable "fishy" odor, with an odor of ammonia, (4), ovaries, in the female crab, watery and the muscle meat disintegrated, a strong ammonia-like odor, (5), a strong "putrid-like" odor, meat dark, completely disintegrated and slimy in appearance. The meat

at this stage is quite alkaline to phenol red, and the ammonia content is quite high as shown by Nessler's reagent.

Total counts. Total aerobic plate counts on ten samples of crabs varied widely. This was to be expected since different crabs were necessarily used for each determination. The highest count for the portion consisting of the stomach and intestines was 9,000,000 organisms per gram; the lowest count being 4,000,000. The average count for this portion for all ten samples was 6,450,000.

For the other internal organs, exclusive of stomach and intestines, the highest count was 14,000,000 bacteria per gram; the lowest, 2,500,000. The average count for all ten samples was 8,150,000 bacteria per gram.

Daily total count determinations of decomposing crabs at 20° C. were higher, on the whole, than those at 37° C. The initial count at 20° C. in a typical sample for the stomach and intestines portion was 8,000,000 bacteria per gram; for the other portion, consisting of the other internal organs, it was 12,000,000. The initial count at 37° C., however, for the same two portions was 750,000 for the former and 10,000,000 for the latter. Total counts at 37° C. on this sample showed that the first peak in the bacterial population was reached after one day of storage with a count of 20 million bacteria per gram for the stomach and intestines portion and 95 million for the other portion. The total counts then declined until the fourteenth day when the maximum counts for both portions were reached, being 115 million bacteria per gram for the former and 100 million for the latter.

Counts at 20° C. on the same two portions of this sample showed that the first peak in bacterial numbers was reached on the third day of storage with 50,000,000 bacteria per gram for the stomach and intestine portion and 80,000,000 for the other internal organs. The total counts then declined until the fourteenth day when they reached their maximum of 1,000,000,000 bacteria per gram for the former and 850,000,000 for the latter.

There seems to be no constant relationship between the period of storage and total bacterial count. The total aerobic plate counts on the twenty-eighth day of storage on the above sample, for example, were lower than the counts on the first and second days. Yet at this time (after 28 days storage) the crabs were in an advanced stage of decomposition.

Spoilage of crab meat. Fresh crab meat has a pleasant characteristic odor and appears sound and whole with a pearly-gray color.

It is comparatively dry. At the end of twenty-four hours storage at room temperature definite signs of spoilage appear. The crab meat is usually slightly darker in color and possesses a somewhat strong "sour" odor. At the end of two days it is still darker in color, a tan-brown, with liquefaction starting. An ammonia-like odor is also noticeable. By the end of the third day liquefaction and darkening are still more advanced and the ammonia-like odor is quite strong. By the sixth day decomposition is much advanced, the meat is quite disintegrated and liquefied and there is a very strong ammonia-like odor. By the eighth day the crab meat is entirely disintegrated, being a dark, dirty-brown "mushy" mass. At no time during spoilage was there any evidence of gas bubbles. Eliot (18) found that in the liquor of decomposing shucked oysters bubbles of gas are abundant at the end of forty-eight hours.

Total count changes. Total count determinations made at 37° C. of twenty samples of crab meat varied widely. The lowest aerobic count was 500,000 organisms per gram, while the highest was 5,000,000 per gram. The average count at 37° C. on these twenty samples was 1,912,500 bacteria per gram.

Counts at 20° C. on the same samples gave different results. The lowest aerobic count was 650,000 while the highest was 7,000,000 per gram. The average count at 20° C. on these twenty samples was 2,245,750. This is somewhat higher than the average count at 37° C. and is in agreement with the results of Cary (11) who found the total aerobic counts of sausage to be higher at 20° C. than at 37° C.

Total counts made on successive days reveal a sudden rise on the first to fourth days which is sometimes the maximum count. If the total counts are made for nine or ten successive days only, the initial rise in the bacterial flora on the first few days may reach its highest point then with a more or less sudden and irregular decline in numbers. If, however, the total aerobic counts are determined over a longer period of time, for 20 to 35 days, we may get a subsequent second or even third rise in the bacterial population after the first decline, which in some samples may even be higher than the first peak.

Relation of temperature to changes in the Bacillus coli scores. If a sample of crab meat is divided into two equal portions, one portion kept at ice-box temperature, the other at room temperature, the daily scores of these two portions vary a great deal. In a typical sample the scores for the portion spoiling at room temperature rise to enormous figures, they may be 4,000,000 or over on the sixth or seventh day. The scores then drop gradually to about the tenth or

twelfth day after which they are zero. The scores for the other portion, on the other hand, are usually low. They never rise above a score of 50.

In comparing the results obtained on two such portions of the same sample of crab meat, it is evident that members of the *Bacillus coli* group do not multiply or are killed at ice-box temperature. If such were not the case, the scores for the portion spoiling at room temperature and for the portion spoiling at ice-box temperature would be the same. Pure cultures of *Bacillus coli* isolated from crab meat do not grow at ice-box temperature.

Changes in acidity. Fresh crab meat is slightly alkaline in reaction, the pH value varying from 7.0 to 7.6 and usually from 7.2 to 7.4. During the first few days of spoilage the pH may not vary much from the original determination; but as decomposition progresses there is a more or less steady rise in pH so that at the end of ten to fifteen days of spoilage the pH is usually between 8.2 and 8.6.

There was only one notable exception to this general rule and this was in a sample of crab meat that had been sterilized in the autoclave and was then inoculated with 1 cc. of a twenty-four hour culture of *Proteus vulgaris*. The initial pH of this particular sample was 7.0 and after six days at room temperature the pH dropped to 6.8. This was the only sample in which the pH dropped below 7.0. On the tenth day the pH was 7.2.

In all other samples which were not sterilized but were allowed to decompose as they were, the alkalinity steadily rose. This is in contrast to the findings of Hunter (29), and Eliot (18) on the pH changes in spoiling shucked oysters. Eliot (18) found that during the first few days of spoilage of shucked oysters the acidity steadily increased so that in one sample the low pH of 4.4 was reached after decomposing for ten days at room temperature.

The difference in acidity changes in decomposing crabs and oysters probably lies in the fact that oysters have more than three times as much carbohydrate as crabs with the result of a more prolonged and higher acid fermentation, whereas crabs contain almost three times as much protein material as oysters with the consequent alkaline reaction resulting.

Ammonia production. A test for spoilage. Indol production. When 0.1 cc. of a 1-10 dilution of fresh crab meat is tested for ammonia with one drop of Nessler's reagent a + test (light yellow color) or no test at all results. After one day's spoilage at room temperature such samples usually show a ++ (dark yellow color) or +++ test

(brown color). If the test is made daily for ten or fifteen days on spoiling crab meat it continues to give a +++ reaction. Ammonia can be detected in spoiling crab meat before other signs of spoilage are evident. In brief this simple test may serve in public health laboratories as a means of differentiating fresh from decomposed crab meat. The test may be described briefly as follows: if upon the addition of one drop of Nessler's reagent to one tenth cubic centimeter of a 1-10 dilution of crab meat a + test or no test results the meat is fresh, if a ++ or +++ test results the meat is spoiled.

Indol production during spoilage of crab meat is much slower than ammonia formation. The first good positive test for indol (Ehrlich method) is not evident before three or four days of spoilage, while ammonia can be detected during the first day of decomposition. After the first positive test for indol the sample always gives a positive test on the following days.

Decomposition of sterilized crab meat by Proteus vulgaris. Sterilized (autoclaved) crab meat inoculated with 1 cc. of a twenty-four hour culture of *Proteus vulgaris* shows definite signs of spoilage by the second day of storage at room temperature. The meat is slightly darker in color at this time and is more moist in appearance. By the third day the muscle meat has begun to disintegrate and has a dirty-brown color. By the end of the tenth day of storage at room temperature such meat is completely disintegrated and watery.

There is a close similarity between the spoilage of a sterilized sample of crab meat by *Proteus vulgaris* and the decomposition of ordinary non-sterilized crab meat by the "natural" flora present. In both appearance and color a sterilized sample inoculated with *Proteus vulgaris* and an ordinary non-sterilized sample when allowed to decompose for ten days cannot be distinguished from each other. There is, however, a difference in odor between two such samples which is probably accounted for by the delay in ammonia and indol production in the former (inoculated meat).

Relation of temperature to total count and pH value in decomposing crab meat. Total counts for portions of crab meat spoiling at room temperature are higher, on the whole, than counts for portions of the same sample spoiling at ice box temperature. This holds true up to about the seventh day of spoilage. After about the seventh day total counts for portions spoiling at ice-box temperature are higher than the counts for the other portion for the corresponding period of storage.

There is no close correlation between hydrogen ion concentration

and total counts. The hydrogen ion concentration, for example, from the eighth to the twenty-sixth day of spoilage may be practically the same whereas the total counts during this period vary enormously. The only general correlation is that as spoilage progresses the hydrogen ion concentration falls.

Correlation between total counts by the agar plate and broth dilution methods. The correlation between total counts by the agar plate method and by the broth dilution method for portions of crab meat spoiling at ice-box temperature is not good. The total counts by the broth dilution method are always lower on these portions than by the plate method. The peak of the bacterial population on these portions as shown by the broth dilution method is usually evident three or four days later than the peak shown by the agar plate method.

The correlation between counts by both methods on portions spoiling at room temperature is somewhat better. Up to about the fifth day of spoilage the total counts by both methods are practically the same. After about the fifth day the total counts by the agar plate method are somewhat higher than the counts by the broth dilution method.

The difference in the results obtained by the two methods is probably due to the fact that the agar plates allow the facultative anaerobes to grow in the depths of the agar. In the broth tubes, on the other hand, there is practically no anaerobiasis and since the streptococci which make up a considerable part of the flora are facultative anaerobes the conditions for growth in the broth tubes are not optimum for them, hence the lower counts by the broth dilution method.

Succession of types of bacteria during spoilage. The succession of types of bacteria during spoilage may be said in brief to be as follows: The streptococci appear early and are continually present throughout the decomposition increasing in numbers as spoilage progresses. Lactose fermenters are present only during the early stages of spoilage. The non-lactose fermenters also appear early and usually decrease in numbers by the tenth or twelfth day. The cocci and sarcinae usually show an early appearance but are not present in large numbers and are encountered only occasionally. These two types as well as the zopfia appear irregularly. The achromobacteria appear rather irregularly during the first eight to twelve days of spoilage, but in considerable numbers. The flavobacteria and pseudomonas appear in rather small numbers and at irregular intervals during the early stages of decomposition.

Toxicity experiments. A watery extract of a twelve-day decomposed sample of crab meat, sterilized by filtration, upon repeated intraperitoneal injections of relatively large doses into several guinea pigs and rats produced no apparent gross effects on these test animals. The bacterial flora and *Bacillus coli* of this sample were known to have reached enormous figures and if toxic substances had been elaborated they would have been evident upon intraperitoneal injections. Since symptoms of any kind were not produced it seems that no substance of either a toxic or anaphylactic nature, for these test animals at least, were present in the watery extract.

Savage (52) has reported some interesting experiments of a comparable nature on the toxicity of certain foods including putrid beef, canned crayfish, crab and salmon. Using kittens as the experimental animals he both fed and injected them subcutaneously with sterile filtrates of these foods which were in such an advanced state of putrefaction that they were obviously unfit for human consumption. On the whole his results were negative. Even though the kittens were subjected to repeated large doses no toxic effect or gross pathological changes were induced. Putrid meat extracts containing both the bacteria and their products were fatal to two rabbits by subcutaneous inoculation. A rabbit fed with the same material remained unaffected. All the animals used were given enormous doses daily for many days with little disturbance of nutrition or loss of weight.

Eliot (18) found that oysters in the shell which had been allowed to pass through all the stages of decomposition in which they would ordinarily be eaten were not toxic to two persons who consumed them. These oysters were given a minimum amount of heating before they were eaten.

Spoilage of crabs and crab meat. The decomposition of crabs and crab meat is a progressive proteolysis of the meat accompanied by a rapid and continuous rise in the ammonia content and a more or less irregular rise in alkalinity. The term decomposition as applied to the spoilage of crab meat is used in a restricted sense, namely, as a proteolytic process without the production, necessarily, of foul odors characteristic of cadaveric putrefaction. "Decomposition," therefore, is to be distinguished from "putrefaction" as defined by Bienstock (56) and Rettger (49).

The decomposition of crabs and crab meat is brought about through the activity of certain aerobic species of bacteria. This in general corroborates the findings of Hunter (28), Eliot (18), Dormeyer (14), and Hildebrand and Bischoff (25). Bischoff found that the

decomposition of fish is brought about by aerobic bacteria only. Obligate spore-forming anaerobes of the *Bacillus putrificus* type were never found by him in fish except in rare instances. Although Eliot (18) found four groups of spore-forming anaerobes in the spoiling oyster, they apparently did not take part in the actual decomposition processes. They were responsible for what Eliot termed the "gaseous state" in the decomposition of oysters.

Attempts were made on a number of samples of crabs and crab meat to isolate obligate or even facultative spore-forming anaerobes but without success. Streptococci were the only types obtained and they corresponded in all respects to those isolated by aerobic methods. All the streptococci encountered, apparently, were facultative anaerobes since they grew best under partial anaerobic conditions.

The decomposition of crab meat differs from that of shucked oysters as found by Eliot (18) in that there are no acid and gaseous stages. It is merely a progressive proteolysis with an irregular rise in alkalinity. The decomposition of crab meat differs from the putrefaction of meats from warm blooded animals in that anaerobes of the "*putrificus*" and "*sporogenes*" types are not present. In meats from warm blooded animals spore-forming anaerobes play a major role in the putrefaction.

The group of aerobes responsible, for the most part, for the decomposition of crab meat is the *Proteus* group. *Proteus vulgaris* in pure culture alone can bring about a rapid and progressive proteolysis of autoclaved crab meat.

Bacteria of the crab. The predominating types of bacteria in the crab are the Gram-negative non-spore forming rods and the Gram-positive streptococci. Chromogenic organisms belonging to the genera *Pseudomonas*, *Flavobacterium*, *Micrococcus*, and *Sarcina* were conspicuous by their low prevalence. They were never found in large numbers in any of the samples. They were present in larger numbers relatively in the crab than in crab meat. Streptococci, although apparently not taking an active part in the decomposition, were always present, particularly during the latter stages of spoilage. After eight to ten days of spoilage they practically dominated the flora. It is quite probable that the strict aerobes, such as the *Proteus* group, during the first five to ten days of spoilage, utilize the dissolved oxygen in the meat and thus afford an especially favorable environment for the multiplication of the streptococci since they were found to grow best under partially anaerobic conditions.

Fifty cultures belonging to the genus *Escherichia* were isolated

and studied. Forty-seven were identified as *Bacillus coli communior* while the other three were the "*communis*" variety. Apparently the sucrose fermenting type of *B. coli* predominates in the crab.

Four groups belonging to the genus *Pseudomonas* were isolated. They all produced acid in glucose and hydrogen sulphide in lead acetate agar only after at least four days incubation at room temperature. The first two groups A and B liquefied gelatin and peptonized milk, group B differing from A in forming indol. Groups C and D neither liquefied gelatin nor peptonized milk. Group C reduced nitrates while D did not. In pure culture groups A and B produce a viscous green growth on autoclaved crab meat and digest the meat slightly after a week or ten days. The members of the other two groups have no proteolytic action on crab meat. The first two groups may take some part in the decomposition of crabs and crab meat.

Five groups of bacteria belonging to the genus *Flavobacterium* were isolated. The members of only one group produced a slight proteolysis of crab meat after ten days.

Eight types of the genus *Achromobacter* were isolated. These did not correspond to any of the varieties described in Bergey's Manual. In pure culture they had no proteolytic action on crab meat.

Two types of the genus *Proteus* were isolated. The first was the typical *Proteus vulgaris* while the second differed in fermenting salicin with acid and gas and not attacking glycerol. The *Proteus* group is the most important single group responsible for the decomposition of crab meat.

Of the genus *Alcaligenes* two types were isolated. The first corresponded to *Alcaligenes fecalis* while the second was unidentified.

Two species of *Zopfius* were isolated. Most strains were identified as *Zopfius zopfii* while other strains differed in producing acid in glucose and galactose.

Five species of the genus *Micrococcus* were isolated and studied. They were identified as *Micrococcus candidus*, *M. flavus*, *M. aurantiacus*, *M. candicans*, and *M. varians*.

Three types belonging to the genus *Sarcina* were found. The first corresponded to *Sarcina flava*, the second to *Sarcina aurantiaca*, and a third was unidentified.

Many strains of streptococci were isolated and studied. After six to seven days of spoilage they practically dominated the flora. They were all facultative anaerobes. They could not be identified with any of the species described in Bergey's Manual.

Conclusions

1. The decomposition of crabs and crab meat is a progressive proteolysis of the meat accompanied by a rapid and continuous rise in the ammonia content.*

2. As decomposition progresses there is a more or less irregular rise in the alkaline reaction.

3. The decomposition is due primarily to the action of the *Proteus* group of organisms, possibly supplemented by some of the *Pseudomonas* and *Flavobacterium* groups.

4. There is no relation between the "age" of decomposition and the total aerobic counts.

5. There is no close relationship between "age" of decomposition and the pH value.

6. Spore-forming anaerobes are not concerned in the decomposition.

7. Samples spoiling at room temperature show a rapid rise and high *Bacillus coli* score.

8. Samples spoiling at ice-box temperature have a low score.

9. Samples spoiling at room temperature show a sudden and maximum rise in the total aerobic count from the first to the fourth days.

10. Samples spoiling at ice-box temperature also show a sudden initial rise in the bacterial population, but the maximum total aerobic count is reached on about the fifteenth to the twentieth day.

11. Bacteria belonging to the following genera have been isolated; *Escherichia*, *Proteus*, *Zopfius*, *Alcaligines*, *Flavobacterium*, *Achromobacter*, *Pseudomonas*, *Micrococcus*, *Sarcina* and *Streptococcus*.

12. Total aerobic counts by the agar plate method are higher than counts by the broth dilution method.

13. The Nessler ammonia test is tentatively proposed as a means of differentiating fresh from spoiled crab meat, before macroscopic signs of spoilage appear. This test may be described briefly as follows: if upon the addition of one drop of Nessler's reagent to 0.1 cc. of a 1-10 dilution of crab meat a + test (light yellow color) or no test results the meat is fresh, if a ++ (dark yellow color) or +++ test (brown color) results the meat is spoiled.

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